INHIBITION OF GLUTATHIONE PEROXIDASE AND THE EFFECT ON LIPID AND MYOGLOBIN OXIDATION IN BEEF

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Abstract – The objective of this study was to investigate the role of glutathione peroxidase (GSH-Px) in relation to myoglobin and lipid oxidation in beef through inhibition of its postmortem activity. Mercaptosuccinate, a specific inhibitor for GSH-Px, was added to minced beef samples (mixture of forequarter muscles) at the concentrations of 0, 0.1, 0.2 and 0.4 mM. Activities of GSH-Px and other antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT) and TBARS (lipid oxidation) and colour values were determined at day 0, 6 and 12 of display. The activity of GSH-Px was reduced with increasing concentrations of mercaptosuccinate ($P<0.001$). Concomitantly, increasing TBARS values were observed with increasing concentrations of the inhibitor ($P<0.001$). Metmyoglobin formation was also higher during the first two days of display when the inhibitor was added compared to the control ($P<0.001$). The SOD and CAT activities were not inhibited ($P>0.05$). It was concluded that GSH-Px plays an important role in retarding lipid oxidation and might also be involved in reducing myoglobin oxidation in beef during postmortem display.

Key Words – Antioxidant enzyme, GSH-Px, Meat, Mercaptosuccinate.

I. INTRODUCTION

Oxidative deterioration in meat leads to a loss of nutritional value and reduced sensory quality. There are several mechanisms to protect muscle in vivo and postmortem against oxidative processes, including the endogenous antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). SOD is the first line antioxidant enzyme defending against reactive oxygen species (ROS) and free radicals, while CAT and GSH-Px reduce hydrogen peroxide and alkyl hydroperoxides in the cytoplasm [1].

Glutathione peroxidase (GSH-Px) is a selenium-containing enzyme, catalyzing the reduction of lipid and hydrogen peroxides to less harmful hydroxides. However, its importance in retarding the oxidative deterioration of muscle postmortem is not well established. It may be argued that a variation in the activity of this enzyme may affect postmortem muscle oxidative stability. For CAT, Pradhan *et al.* [2] has demonstrated that this enzyme seems to play an important role in modulating lipid oxidation in postmortem muscle from different species.

A number of mercaptocarboxylic acids and tertiary mercaptans are known to be strong and specific inhibitors of the enzyme GSH-Px. Chaudiere *et al.* [3] stated that three of the most potent inhibitors for GSH-Px are mercaptosuccinate, penicillamine, and α-mercaptopropionylglycine. Mercapto-succinate appears to be the most effective inhibitor in this series and may not affect other biological components to a significant extent.

Hence, the objectives of this study were 1) to determine the effect of mercaptosuccinate ($C_4H_6O_4S$) on the activity of GSH-Px and other antioxidant enzymes (SOD and CAT) in minced beef during postmortem display and 2) to investigate the effect of this inhibition on lipid and myoglobin oxidation.

II. MATERIALS AND METHODS

Preparation of meat samples

Muscles from the forequarter of a young Belgian Blue bull were taken at 48 hour postmortem. The beef samples were stored frozen until use. The frozen meat was minced and mixed thoroughly with the inhibitor solution at a concentration of 2.5%. Mercaptosuccinate ($C_4H_6O_4S$; Sigma-Aldrich) was dissolved in 0.9% NaCl and was used as inhibitor at a final concentration of 0, 0.1, 0.2 and 0.4 mM in meat. After mixing, 50 g meat patties were made and divided in 3 groups according to the 3 time points of display with 2 replicates per time point. The patties were

wrapped with oxygen permeable foil and displayed at 4°C under fluorescent light (approximately 1200 lux). Samples were removed for analysis after 0, 6 and 12 days of display.

Antioxidant enzyme activity assays

During these analyses, the muscle samples were kept on ice. A 5 g sample was homogenized in 10 ml of 0.05 M phosphate buffer (pH 7.0) and centrifuged at 4°C for 20 min at 7000g. The supernatant fraction was filtered through glass wool before determining enzyme activities.

The activity of GSH-Px was determined by measuring the oxidation of NADPH. One unit of GSH-Px activity was defined as the amount of extract required to oxidize 1 µmol of NADPH per min at 25°C [4]. The SOD activity assay was performed as described by Marklund and Marklund [5] by measuring the inhibition of pyrogallol autoxidation. A unit of enzyme activity was defined as the amount of sample needed to inhibit the reaction by 50%. The CAT activity was determined according to the method of Aebi [6]. One unit of CAT activity was defined as the amount of sample required to decompose 1 µmol of H_2O_2 per min at room temperature.

Thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was assessed spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method based on Tarladgis, Watts & Younathan [7] and is expressed as µg malondialdehyde (MDA) per g meat.

Colour values and metmyoglobin formation

Colour *L**, *a**, *b** and reflectance values were measured with a Hunterlab Miniscan colour meter (D65 light source, 10° standard observer, 45°/0° geometry, 1-inch light surface, white standard) at 0, 1, 2, 5, 6, 7, 8, 9 and 12 day of display. The % metmyoglobin (%MetMb) was calculated from the reflectance values at specific wavelengths [8].

Statistical analysis

The data were analyzed by General Linear Model procedures to test for the effect of inhibitor concentration and day of display by using the SAS software, followed by Tukey post-hoc multiple comparison of means tests in case of significance.

III. RESULTS AND DISCUSSION

Antioxidant enzyme activity inhibition

The activity of GSH-Px decreased with increasing concentrations of mercaptosuccinate at the three time points ($P<0.001$) (Table 1). The relative inhibition compared to the control was 17%, 27% and 42% at 0.1, 0.2 and 0.4 mM respectively. This effect is in line with Chaudiere *et al.* [3] who found that mercaptosuccinate inhibited GSH-Px activity in hamster liver, and this inhibition was not pH-dependent around neutral pH. Their results also supported the formation of reversible enzyme-inhibitor complexes. The active site selenium is trapped by the rapid binding of the inhibitor in competition with GSH. At day 0 and 6 of display, mercaptosuccinate had no effect on CAT and SOD activities ($P>0.05$). Only at day 12 of display, mercaptosuccinate at 0.1 and 0.4 mM resulted in slightly higher activities of CAT and at 0.2 mM in a slightly higher activity of SOD compared to the control ($P<0.05$). Mercaptosuccinate thus appears to be a specific inhibitor of GSH-Px in muscle.

Across inhibitor treatments, the activities of GSH-Px, CAT and SOD were lower at day 6 and 12 of display compared to day 0 ($P<0.001$), with no difference between day 6 and 12 for GSH-Px and SOD, and surprisingly a higher CAT activity at day 12 compared to day 6. Renerre *et al.* [9] also reported a decrease in GSH-Px and SOD activity in different beef muscles until day 6 postmortem. However, these authors did not find a decrease in CAT activity with time in contrast to our findings.

Thiobarbituric acid reactive substances (TBARS)

As expected, TBARS values increased with time and were higher at day 6 and 12 of display compared to day 0 ($P<0.001$). In line with the reduction in GSH-Px activity, TBARS values increased with increasing concentration of mercaptosuccinate ($P<0.001$; Table 1). After 6 days of display, TBARS values were 10%, 23% and 46% higher for 0.1, 0.2 and 0.4 mM inhibitor respectively compared to the control.

Table 1 Effect of Mercaptosuccinate on CAT, SOD and GSH-Px activities and TBARS values in minced beef samples during display (mean ± SD)

		Mercaptosuccinate concentration			
	day	Control	0.1 mM	0.2 mM	0.4 mM
GSH-Px	0	1.15±0.05^{a,x}	0.94±0.02^{b,x}	0.85±0.01^{c,x}	0.72±0.04^{d,x}
(U/g)	6	0.86±0.03^{a,y}	0.78±0.01^{b,y}	0.73±0.00^{c,y}	0.62±0.01^{d,y}
	12	1.08±0.13^{a,x}	0.84±0.08^{b,y}	0.69±0.07^{b,y}	0.48±0.04^{c,z}
CAT	0	138.01±8.23^{x}	130.73±10.77^{x}	136.65±9.94^{x}	134.25±7.44^{x}
(U/g)	6	77.01±10.42^{y}	77.57±5.13^{z}	74.57±2.80^{z}	77.35±7.41^{z}
	12	90.32±3.07^{b,y}	105.77±1.07^{a,y}	92.59±7.27^{b,y}	107.16±4.36^{a,y}
SOD	0	59.20±2.02^{x}	63.10±1.28^{x}	61.17±0.94^{x}	62.51±3.53^{x}
(U/g)	6	40.39±0.25^{y}	40.41±1.24^{y}	41.65±3.76^{y}	46.47±3.36^{y}
	12	39.49±0.47^{b,y}	40.54±0.38^{ab,y}	39.05±0.35^{b,y}	41.15±0.29^{a,y}
TBARS	0	0.34±0.13^{b,y}	0.35±0.02^{b,z}	0.43±0.04^{ab,z}	0.57±0.02^{a,y}
(µg/g)	6	6.37±0.09^{c,x}	7.04±0.44^{c,y}	7.81±0.27^{b,y}	9.27±0.40^{a,x}
	12	6.33±0.19^{c,x}	7.69±0.10^{b,x}	8.64±0.24^{a,x}	9.06±0.26^{a,x}

^{a,b,c,d} Within a row, mean values with different superscripts differ significantly at P<0.05

^{x,y,z} Within a column and variable, mean values with different superscripts differ significantly at P<0.05

Table 2 Effect of Mercaptosuccinate on %MetMb in minced beef samples during display

	Mercaptosuccinate concentration			
Day	control	0.1 mM	0.2 mM	0.4 mM
0	19.4±0.4	19.4±0.4	19.4±0.3	19.4±0.2
1	29.1±0.5^{c}	29.5±0.3^{c}	32.6±0.6^{b}	34.5±1.0^{a}
2	35.9±0.3^{d}	38.8±0.5^{c}	42.2±0.9^{b}	45.6±0.6^{a}
5	59.1±0.8^{a}	57.4±0.7^{b}	55.5±0.5^{c}	51.1±0.8^{d}
6	59.9±1.6^{a}	58.2±1.6^{a}	57.7±0.8^{a}	52.5±1.6^{b}
7	63.0±2.8	62.1±0.2	58.3±0.1	59.8±0.4
8	62.5±0.3	62.4±3.7	61.3±0.1	61.5±0.4
9	65.9±1.5^{ab}	64.5±1.8^{ab}	61.8±0.7^{b}	67.7±1.3^{a}
12	50.6±0.4^{b}	61.1±6.7^{ab}	67.2±1.0^{a}	66.7±0.3^{a}

^{a,b,c,d} Within a row, mean values with different superscripts differ significantly at P<0.05

Colour values and metmyoglobin formation

The average *L**, *a** and *b** values at day 0 were 48.8±1.0, 23.9±0.4 and 21.6±0.8 respectively. The average *L** value strongly decreased during the first day of display (*L** at d1: 40.1±0.4) and increased slightly during further display. The addition of mercaptosuccinate to the minced beef resulted in higher *L** values compared to the control until day 8 of display (P<0.05; except for day 1).

The *a** value was not different between treatments at day 0 and 1 of display and strongly decreased during the first day of display (*a** at d1: 14.4±0.3). The *a** values further decreased during display and were slightly lower for the mercaptosuccinate treatments compared to the control at day 2, 5 and 7 of display (P<0.05). These differences were, however, rather limited.

The formation of metmyoglobin steadily increased from day 0 until day 12 of display in all treatments (Table 2). The initial %MetMb at day 0 was not different between treatments (P>0.05). At day 1 and 2 of display, %MetMb was higher in the 0.2 and 0.4 mM mercaptosuccinate treatment compared to 0 and 0.1 mM mercaptosuccinate (P<0.05). In contrast, at day 5 and 6 of display, %MetMb in 0.4 mM mercaptosuccinate samples was lower than for the other treatments (P<0.05).

These data suggest that GSH-Px is involved in reducing myoglobin oxidation, at least during the initial period of display. However, the colour data have to be interpreted carefully since the addition of the inhibitor appeared to affect the colour.

IV. CONCLUSION

The postmortem activity of GSH-Px in meat can be inhibited by mercaptosuccinate in a specific and concentration dependent manner. Inhibiting the GSH-Px activity increased lipid oxidation and the initial formation of metmyoglobin. This suggests that GSH-Px has an important role in retarding oxidation in fresh meat during storage.

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